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Effect of Phenol Molecular Structure on Bacterial Transformation Rate Constants in Pond and River Samples

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Microbial transformation rate constants for a series of phenols were correlated with a property of the substituents, van der Waal's radius. Transformation products were the corresponding catechols, with the exception of *p*-hydroxybenzoic acid, the product of *p*-acetylphenol. A different product suggested a different pathway; *p*-acetylphenol, therefore, was deleted from the data base.

Relationships between microbial rate constants and the physical or chemical properties of a group of congeners have been reported for pesticides, phthalates (12), and phenols (6). In the latter study, microbial (a culture of *Pseudomonas putida* U) rate constants correlated with van der Waal's radius, a property of the substituent of the compounds (6). To be useful in predicting rate constants for the degradation of substituted phenols in natural waters, this approach needs to be evaluated with mixed populations. We therefore extended our studies on the microbial transformation of phenols to environmental waters and developed a relationship between disappearance rate constants and physical properties for a series of these compounds.

Water samples were collected from each of five sites within 10 km of Athens, Ga., transported to the laboratory in sterile glass jugs, and stored at 7°C before use. Studies of process kinetics were generally initiated within 5 h after sample collection; sequential studies were made within 96 h.

The materials used were the same as previously reported (6), except that *p*-hydroxybenzoic acid, obtained from Chem Services, Inc., West Chester, Pa., was used as a standard in the product studies.

Approximately 200 ml of freshly collected water was transferred to four 500-ml flasks. A fifth flask containing 200 ml of filter-sterilized water served as a control for abiotic processes. To the five flasks, a filter-sterilized aqueous solution of the test phenol was added to give a final concentration of 1.0 mg of phenol per liter. The solutions were incubated in a temperature-controlled shaker at 25°C. The concentration of bacteria from the five different sites spanned two orders of magnitude (10^7 to 10^9 organisms per liter). Culture media, enumeration of bacteria, and transformation kinetics were described previously (6).

The intermediate transformation product of all the phenols investigated was the corresponding catechol, with the exception of *p*-acetylphenol. The product was identified as *p*-hydroxybenzoic acid by thin-layer chromatography and comparison of R_f values with authentic samples. In chloroform-ethanol-0.1 N sodium hydroxide (100:5:1) (8) the ratio of the R_f of the acid to that of *p*-acetylphenol was 0.4, whereas in benzene-methanol-acetic acid (45:10:1) (9) the ratio was 0.89. A different product suggested a different pathway; therefore, *p*-acetylphenol was excluded from the correlation of reactivity with chemical structure.

Mean values for the disappearance rate constant for the phenols in waters from five sites are shown in Table 1. The values ranged from 2.5 ($\pm$1.0) $\times$ 10^{-12} liter $\cdot$ organism^{-1} $\cdot$ h^{-1} for *p*-cyanophenol to 3.3 ($\pm$1.2) $\times$ 10^{-10} liter $\cdot$ organism^{-1} $\cdot$ h^{-1} for phenol. For mixed populations and *P. putida* studies, the rate constants of similar compounds were in the same relative order, except for *p*-acetyl-, *p*-nitro-, and *p*-methoxyphenol. The order of the latter two was reversed; however, they were less than a factor of two different from each other in either study. *p*-Acetylphenol, however, was transformed 645 times faster in natural waters than in the pure culture.

Although relative reactivities were similar, rate constants for all the phenols were higher in natural waters than in the pure culture. Bull and Brown (2) and D. L. Lewis (unpublished data) observed this phenomenon for compounds other than phenols.

In an earlier study in our laboratory (7), microbial disappearance rate constants for three pesticides were reproducible from site to site. A comparison of the phenol rate constants found in the present study with data from other researchers gave similar agreement. Visser et al. (11) studied bacteria active in the degradation of

TABLE 1. Microbial transformation rate constants (K_b) for eight phenols and five sites

Compound	K_b (liter · organism^{-1} · h^{-1}) (mean (±SE)[a] at site no.: 1 (Williams Pond)	2 (Overlook Pond)	3 (Park Pond)	4 (Hickory Hills)	5 (Oconee River)	Mean for all sites
Phenol	3.0 (±1.3) × 10^{-10}	4.8 (±3.1) × 10^{-10}	2.4 (±1.2) × 10^{-10}	2.0 (±1.5) × 10^{-10}	4.2 (±2.6) × 10^{-10}	3.3 (±1.2) × 10^{-10}
p-Methylphenol	1.1 (±0.3) × 10^{-10}	4.6 (±2.7) × 10^{-10}	2.4 (±1.4) × 10^{-10}	2.6 (±1.7) × 10^{-10}	3.0 (±1.3) × 10^{-10}	2.7 (±1.3) × 10^{-10}
p-Methoxyphenol	1.1 (±0.7) × 10^{-11}	1.1 (±0.9) × 10^{-11}	3.5 (±1.4) × 10^{-11}	1.7 (±0.5) × 10^{-11}	3.1 (±1.7) × 10^{-11}	2.2 (±1.1) × 10^{-11}
p-Chlorophenol	8.4 (±1.7) × 10^{-11}	4.7 (±1.6) × 10^{-11}	6.8 (±2.5) × 10^{-11}	7.0 (±2.4) × 10^{-11}	9.0 (±1.7) × 10^{-11}	7.1 (±1.6) × 10^{-11}
p-Bromophenol	9.6 (±2.3) × 10^{-11}	9.6 (±2.4) × 10^{-11}	7.9 (±1.7) × 10^{-11}	9.9 (±1.3) × 10^{-11}	8.2 (±1.8) × 10^{-11}	9.1 (±1.0) × 10^{-11}
p-Acetylphenol	1.8 (±0.8) × 10^{-10}	2.0 (±0.8) × 10^{-10}	4.1 (±2.3) × 10^{-10}	2.1 (±1.4) × 10^{-10}	1.1 (±0.4) × 10^{-10}	2.0 (±1.0) × 10^{-10}
p-Cyanophenol	2.5 (±1.0) × 10^{-12}	2.4 (±1.8) × 10^{-12}	4.2 (±2.5) × 10^{-12}	4.8 (±2.2) × 10^{-12}	6.9 (±2.1) × 10^{-12}	4.2 (±1.7) × 10^{-12}
p-Nitrophenol	4.8 (±0.5) × 10^{-11}	1.7 (±0.9) × 10^{-11}	4.1 (±1.8) × 10^{-11}	3.3 (±2.4) × 10^{-11}	5.2 (±2.6) × 10^{-11}	3.8 (±1.4) × 10^{-11}

[a] Mean value of eight determinations per site with the standard error of the estimate.

phenols in polluted waters of the St. Lawrence River. The calculated disappearance rate constant for phenol from their results was 3.0×10^{-10} liters · organism^{-1} · h^{-1}. The rate constant value for phenol in this study, 3.3×10^{-10} liter · organism^{-1} · h^{-1}, was similar.

From the pure culture study it was anticipated that the microbial rate constants determined here (Table 1) for pond and river populations and van der Waal's radii would correlate. Using mean values of K_b (Table 1) (excluding *p*-acetylphenol), 91% of the variance of the data could be accounted for by van der Waal's radii (Fig. 1).

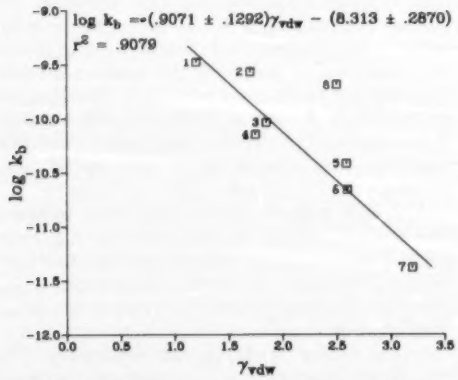

FIG. 1. Correlation of microbial degradation rate constants with van der Waal's radii. Compounds: 1, phenol; 2, *p*-methylphenol; 3, *p*-chlorophenol; 4, *p*-bromophenol; 5, *p*-nitrophenol; 6, *p*-methoxyphenol; 7, *p*-cyarophenol; and 8, *p*-acetylphenol, which was not used in the correlation.

The *F* value (19.72) indicated that the correlation was highly significant. Values (± standard errors) of *m* and *b* were −0.907 ± 0.13 and −8.31 ± 0.29, respectively. The relatively large slope (*m*) for both pure and environmental studies reflected the sensitivity of K_b values to change in the substituent as expressed by the van der Waal's radii. The larger value for the intercept (*b*) showed that the rate constant was about an order of magnitude greater in the environmental waters than in the pure culture.

Other steric properties (Table 2) were examined as well in hopes of decreasing the residual error. Low regression coefficients, however, were obtained when these properties were related to microbial reactivity. The question then was how well the values of these structural properties related to each other. In the correlation matrix (Table 2), the strongest intercorrelations were between bulk steric properties—molecular volume and surface area ($r^2 = 0.952$) and molecular volume and molar refractivity ($r^2 = 0.921$). The width steric parameters, E_s and γ_{vdw}, had a low regression coefficient ($r^2 = 0.645$). When values for the methoxy group were omitted, however, the regression coefficient was higher ($r^2 = 0.989$). In computing E_s for the methoxy group, a value of 1.5, which is the γ_{vdw} for oxygen, was used for van der Waal's radius, whereas the van der Waal's radius used here was 2.6. If it is assumed that the methoxy group is rigid, like the nitro group (5), and cannot rotate to find a position of minimum steric action, then the higher value (2.6) would be a better fit.

A relationship between γ_{vdw} and microbial

TABLE 2. Correlation coefficients among structural parameters[a]

Parameter	r^2 with parameter:							
	γ_{vdw}[b]	E_s[c]	B_4[d]	L[d]	Surface area[e]	Molecular vol[e]	Molar refractivity[f]	Mol wt[f]
γ_{vdw}	1.0	0.645	0.230	0.654	0.466	0.4351	0.297	0.0415
E_s		1.0	0.0066	0.347	0.145	0.175	0.173	0.0372
B_4			1.0	0.322	0.817	0.667	0.519	0.155
L				1.0	0.693	0.802	0.711	0.341
Surface area					1.0	0.952	0.795	0.285
Molecular vol						1.0	0.921	0.454
Molar refractivity							1.0	0.705
Mol wt								1.0

[a] γ_{vdw}, E_s, and B_4 characterize width and L characterizes length of substituent; surface area, molecular volume, molar refractivity, and molecular weight characterize bulk properties.
[b] Reference 6.
[c] Reference 1.
[d] Reference 11.
[e] R. S. Pearlman, personal communication.
[f] Reference 3.

transformation constants has been shown for environmental heterogeneous bacterial populations as well as for pure cultures. These results suggest that this approach may be useful for predicting the rate of transformation of xenobiotics by microorganisms in aquatic systems, as well as for providing some insight into the details of transformation at the mechanistic level. The data base should be broadened to include other microbial xenobiotic transformation processes.

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